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<p align="center">FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III</p>	<p align="center">Issue No. 3</p>
	<p align="center">Effective Date: 6-March-2006</p>
<p>2 DNA PURIFICATION</p> <p>2.1 TECHNICAL NOTES</p> <p>2.1.1 Organic extractions denature and remove the proteins that were released by the proteolytic activity of the Proteinase K. The denatured proteins will collect in the organic phase of the extraction.</p> <p>2.1.2 The interphase may contain some DNA and therefore a minimal amount should be brought along in subsequent organic extractions.</p> <p>2.1.3 The anisotropic, hydrophilic membrane in the Microcon® 50 concentrator is designed to retain molecules that have molecular weights above 50,000 and for the Microcon® 100 concentrator molecules that have molecular weights above 100,000.</p> <p>2.1.4 The Microcon® 100 concentrator is designed to remove low molecular weight contaminants (i.e., peptides and lipids) and inhibitors that may affect the amplification process. Therefore, the Microcon® 100 concentrator is used to purify the sample DNA where the Microcon® 50 concentrator is used for minute samples to concentrate the sample down to a lower volume.</p> <p>2.1.5 The DNA IQ™ System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less than 50 ng of DNA.</p> <p>2.1.6 The DNA IQ™ Lysis buffer is a proprietary detergent mixture containing Guanidine Thiocyanate (GTC). The GTC is a chaotropic agent, necessary for the DNA to stick to the silica-coated, paramagnetic resin.</p> <p>2.1.7 The DNA sticks to the resin through hydrophobic interactions, however the exact mechanism is unknown.</p> <p>2.1.8 The DNA IQ™ Wash Buffer is a low salt buffer, which is 50% alcohol (50% isopropyl: 50% ethanol), and 50 % NaAcetate. The alcohol in the wash keeps the DNA stuck to the resin and the low salt buffer helps to remove excess salt (Guanidine Thiocyanate) from the DNA stuck to the resin. If the Guanidine Thiocyanate is not removed this could interfere with the PCR amplification.</p> <p>2.1.9 The DNA is removed from the resin with DNA IQ™ Elution Buffer using heat (56°C). When the DNA is initially eluted from the DNA IQ™ paramagnetic resin some of it is single stranded and therefore cannot be quantitated on an agarose gel.</p> <p>2.2 EQUIPMENT</p> <p>2.2.1 Pipettes - 20 µL, 100 µL and 1000 µL</p> <p>2.2.2 Microcentrifuge</p> <p>2.2.3 Microcentrifuge tube rack</p> <p>2.2.4 DNA concentrator/evaporator</p>	

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<div> <div>2.2.5 Freezer, -20° C</div> <div>2.2.6 Vortex mixer</div> <div>2.2.7 Heat block or incubator, 56° C</div> <div>2.2.8 Magnet Sphere Stand (Promega Catalog Number Z5342)</div> </div> <div> <div>2.3 MATERIALS</div> <div> <div>2.3.1 Transfer pipettes</div> <div>2.3.2 Microcentrifuge tubes, 1.5 mL</div> <div>2.3.3 Sterile ART tips for pipettes, 20 µL, 100 µL and 1000 µL</div> <div>2.3.4 Microcon® 50 Concentrator Assembly (for concentrating samples)</div> <div>2.3.5 Microcon® 100 Concentrator Assembly (for purifying samples)</div> <div>2.3.6 Kimwipes</div> <div>2.3.7 Gloves</div> </div> </div> <div> <div>2.4 REAGENTS</div> <div> <div>2.4.1 Phenol-chloroform-isoamyl alcohol, prewarmed to room temperature</div> <div>2.4.2 1X TE⁻⁴</div> <div>2.4.3 Sterile Type 1 Water</div> <div>2.4.4 DNA IQ™ Lysis Buffer</div> <div>2.4.5 DNA IQ™ Wash Buffer</div> <div>2.4.6 DNA IQ™ Elution Buffer</div> </div> </div>	

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<p>2.5 MICROCON® PURIFICATION PROCEDURE</p> <p>The Microcon® purification procedure will be used when the biological stain extracted was deposited on a substrate known to cause inhibition during amplification (i.e., denim or velvet), the substrate released an excessive amount of dye during the extraction process, or the biological stain/material was minute in size (e.g., hair root).</p> <p>2.5.1 Add 500 µL phenol-chloroform-isoamyl alcohol, prewarmed to room temperature, to each tube.</p> <p>2.5.2 Cap the tube tightly and mix thoroughly by hand or light vortexing for 2-3 seconds (until solution has a milky appearance).</p> <p>2.5.3 Spin the tube for 3 minutes in a microcentrifuge at approximately 10,000 rpm to separate the two phases.</p> <p>2.5.4 Insert a labeled Microcon® 100 concentrator (or Microcon® 50 concentrator for minute biological stains/material) into a labeled filtrate vial ((microcentrifuge tube provided with the Microcon® assembly); then add 100 µL of sterile Type 1 Water to the concentrator. Using a transfer pipette, transfer the aqueous phase (top layer containing DNA in step 2.5.3) to the Microcon® concentrator. Avoid pipetting organic solvent from the tube into the concentrator. Place the cap from the filtrate vial on the concentrator.</p> <p>2.5.5 Place the Microcon® assembly in a microcentrifuge and spin for 10 to 40 minutes at approximately 5,000 rpm until the volume is reduced.</p> <p>2.5.6 Carefully remove the concentrator unit from the Microcon® assembly and discard the fluid from the filtrate vial. Return the concentrator to the top of the filtrate vial.</p> <p>2.5.7 Add 200 µL of sterile Type 1 Water. Replace the cap and spin the Microcon® assembly in a microcentrifuge at 5,000 rpm for 10-30 minutes until the volume is reduced.</p> <p>NOTE: If the concentrator filter becomes stained due to the dyes released from the substrate, step 2.5.7 may be repeated additional times to reduce the potential of the dye inhibiting the PCR amplification.</p> <p>2.5.8 Remove the cap from the concentrator and add 30 µL 1X TE⁻⁴ buffer. Remove the concentrator from the filtrate vial and discard the vial. Carefully invert the concentrator and place into a new labeled retentate vial (same type of tube as the filtrate vial).</p> <p>2.5.9 Place the Microcon® assembly into a microcentrifuge (the retentate cup end first) and spin at 5,000 rpm for 5 minutes.</p> <p>2.5.10 Discard the concentrator unit; place the cap on the retentate cup.</p> <p>2.5.11 Proceed to Section 4, AluQuant® Human Quantitation System.</p> <p>2.5.12 Refer to Appendix J for the procedure for drying down and resolubilizing extracted DNA samples.</p>	

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<p>2.6 DNA IQ™ MANUAL PURIFICATION PROCEDURE</p> <p>2.6.1 Depending on the type of sample being purified, add the following volumes of DNA IQ™ Lysis Buffer to the sample:</p> <p>NOTE: On the day of use add fresh DTT to the DNA IQ™ Lysis buffer at the concentration described in the reagent preparation section (Appendix B).</p> <p>2.6.1.1 Non-sperm fractions and tissue – Remove 100 µL of the nonsperm fraction/tissue lysate and place it into a new labeled 1.5 mL microcentrifuge then add 220 µL of DNA IQ™ Lysis Buffer. Note: if more than 100 µL of the non-sperm fraction is used add proportional volumes of DNA IQ™ Lysis Buffer.</p> <p>2.6.1.2 Sperm pellet – add 220 µL of DNA IQ™ Lysis Buffer</p> <p>2.6.1.3 Hair samples (optional: envelopes, stamps, cigarette butts, flakes of blood, and other low level samples) – add 220 µL of DNA IQ™ Lysis Buffer. Note: this can be increased proportionally depending on the volume of ProK based digestion buffer used.</p> <p>NOTE: The hair sample/fragment will be dissolved in solution at the completion of the DNA IQ™ isolation procedure.</p> <p>2.6.1.4 Blood Stains and buccal cell type samples – add 100 µL of DNA IQ™ Lysis Buffer</p> <p>2.6.2 Vigorously vortex the bottle of DNA IQ™ Resin for 30 seconds prior to dispensing. Then add 8 µL of the DNA IQ™ Resin to each tube.</p> <p>NOTE: If the stock bottle of DNA IQ™ Resin has set for a prolonged period of time between samples re-vortex before further use.</p> <p>2.6.3 After adding resin vigorously vortex each sample for several seconds</p> <p>2.6.4 Place the tubes in a microcentrifuge rack and allow the samples to set at room temperature for a minimum of 5 minutes to adhere the DNA to the resin. After incubation a brief pulse spin in a microcentrifuge.</p> <p>2.6.5 Transfer the sample tubes to a magnet sphere stand. Open the caps and without disturbing the resin pellet remove the liquid in each tube using a P1000 pipette and discard.</p> <p>2.6.6 Add 100 µL of Lysis buffer into each tube, then remove the tubes from the magnetic stand and vortex vigorously for several seconds. Place the tube in a microcentrifuge and briefly pulse spin.</p> <p>2.6.7 Place tubes back into the magnet sphere stand and without disturbing the resin pellet remove the liquid in each tube using a P100 pipette and discard.</p>	

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<div> <div>2.6.8</div> <div>Add 100 µL of 1X DNA IQ™ Wash buffer to each tube and vigorously vortex for several seconds, followed by a brief pulse spin in a microcentrifuge.</div> </div> <div> <div>2.6.9</div> <div>Place the tubes into the magnet sphere stand and remove the wash buffer using a P100 pipette and discard.</div> </div> <div> <div>2.6.10</div> <div>Repeat steps 2.6.8 and 2.6.9 two additional times.</div> </div> <div> <div>2.6.11</div> <div>After the last wash step, open the cap on each tube and allow the samples to completely air-dry. <u>Note:</u> This will take approximately 5 minutes depending on the volume of liquid remaining in the tube.</div> </div> <div> <div>2.6.12</div> <div>Add 40 µL of the DNA IQ™ Elution buffer to each tube to remove the DNA from the resin. Vortex each tube vigorously for 5 seconds.</div> </div> <div> <div>2.6.13</div> <div>Place the tubes in 56°C heat block for 5 minutes, then in a microcentrifuge and briefly pulse spin.</div> </div> <div> <div>2.6.14</div> <div>Place the tubes into the magnet sphere stand and remove the supernatant using a P100 pipette. DO NOT DISCARD. THE SUPERNATANT CONTAINS THE ISOLATED DNA SAMPLE. IF DNA IQ™ RESIN IS REMOVED WITH THE SUPERNATANT THIS MAY CAUSE INHIBITION AND PREVENT THE SAMPLE FROM AMPLIFYING.</div> </div> <div> <div>2.6.15</div> <div>Place the supernatant into a clean, labeled 1.5 mL microcentrifuge tube.</div> </div> <div> <div>2.6.16</div> <div>Proceed to Section 4, AluQuant® Human Quantitation System.</div> </div> <div> <div>2.6.17</div> <div>Refer to Appendix J for the procedure for drying down and resolubilizing extracted DNA samples.</div> </div> <div> <div>◆END</div> </div>	